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Alkaloid Synthesis |*Hot Paper*|

Collective Synthesis and Biological Evaluation of Tryptophan-Based Dimeric Diketopiperazine Alkaloids

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Abstract: A concise two one-pot synthesis of WIN 64821, eurocristatine, 15,15′-bis-*epi*-eurocristatine, ditryptophenaline, ditryptoleucine A, WIN 64745, cristatumin C, asperdimin, naseseazine A, and naseseazine B is detailed, based on a unique bioinspired dimerization reaction of tryptophan derivatives in aqueous acidic solution and a one-pot procedure for the construction of diketopiperazine rings. Total yields of these alkaloid syntheses were from 10 up to 27%. In addition, 1′-(2-phenylethylene)-ditryptophenaline was synthesized by using three one-pot operations. The studies detailed herein provided synthesized natural products for inhibitory activities of ubiquitin-specific protease 7 (USP7) and foam cell formation in macrophages. The newly listed biological evaluation for tryptophan-based dimeric diketopiperazine alkaloids discovered 15,15′-bis-*epi*-eurocristatine, 1′-(2-phenylethylene)-ditryptophenaline, and WIN 64745 as new drug candidates.

Introduction

A large number of tryptophan-based dimeric diketopiperazine alkaloids has been isolated as secondary metabolites from fungi, such as *Aspergillus*, *Streptomyces*, and *Eurotium* species (Figure 1).[1] Some of these alkaloids show attractive biological activities, such as competitive substance P antagonist activity[2] with respect to the human neurokinin-1 and cholecystokinin B receptor, cytotoxic activity,[3] antibacterial activity,[3] and antiviral activity.[4] A major group of this class of alkaloids contains tryptophan-based homodimeric alkaloids such as WIN 64821[2a] (**1**, also called Q20547A[2e]), eurocristatine[5] (**2**, also called cristatumin E[3]), 15,15′-bis-*epi*-eurocristatine (**3**),[2e] ditryptophenaline

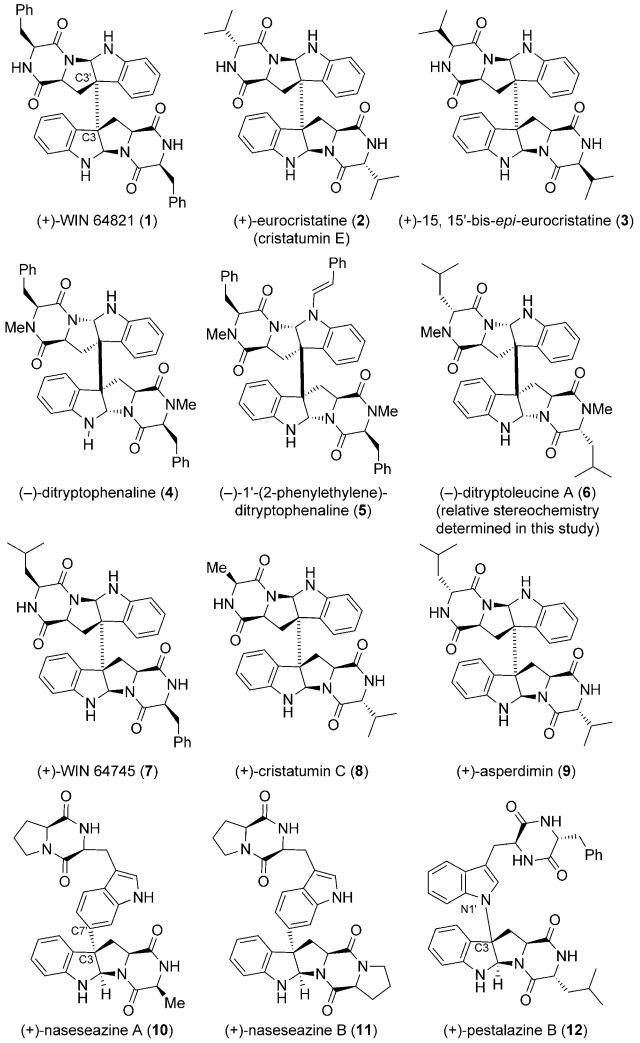

Figure 1. Structures of tryptophan-based dimeric diketopiperazine alkaloids.

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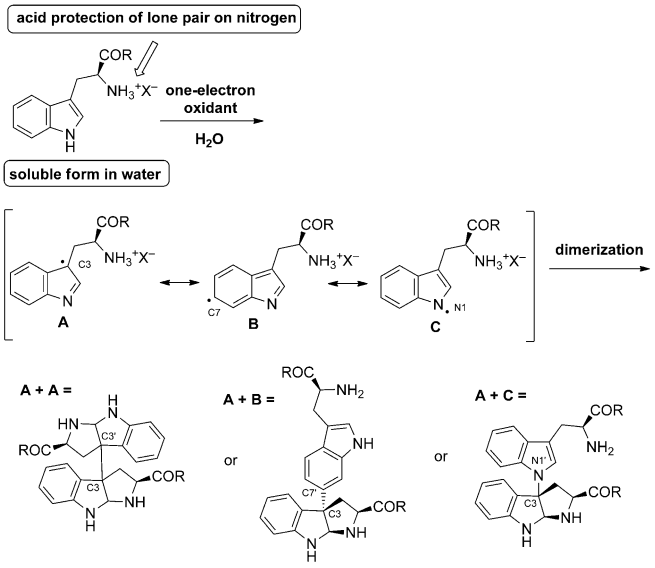
(**4**),[6] 1′-(2-phenylethylene)-ditryptophenaline (**5**),[7] ditryptoleucine A (**6**),[8] WIN 64745 (**7**),[2a] cristatumin C (**8**),[9] and asperdimin (**9**),[10] which possess vicinal quaternary stereocenters joined though C3(sp^3)–C3(sp^3) bonds. On the other hand, a C3(sp^3)–C7(sp^2) bridge has been observed in the structure in naseseazine A (**10**) and naseseazine B (**11**).[11] Furthermore, in 2008, pestalazine B (**12**) possessing a C3(sp^3)–N1 bridge was isolated.[4,12] These alkaloids exhibit a unique architecture derived from an indole oxidation reaction of tryptophan in the biosynthesis and a wide variety of biological activities; accordingly, several biomimetic and non-biomimetic approaches to the synthesis of these alkaloids have been reported.[9b,10b,11b,12,13]

A number of pioneering researchers have adopted a procedure to define bioinspired oxidative coupling reactions of tryptamine or tryptophan units. In 1981, Nakagawa and co-workers reported a bioinspired coupling protocol enlisting a thallium(III)-mediated oxidative dimerization reaction for the first total synthesis of ditryptophenaline (**4**).[13a] Although the yield of the key coupling reaction was not excellent (3% yield), the work made a huge contribution to the recognition of the radical-mediated biosynthetic pathway, the determination of the absolute stereochemistry, and the first attempt at a direct dimerization reaction with an unprotected indole core of a tryptophan derivative. In 2008, Movassaghi and co-workers reported elegant syntheses of WIN 64821 (**1**), ditryptophenaline (**4**), and 1′-(2-phenylethylene)-ditryptophenaline (**5**) through cobalt(I)-mediated bioinspired dimerization reactions with C3-brominated pyrollidinoindoline derivatives.[13e] The method of Movassaghi et al. has been employed for several syntheses of the same class of alkaloids by his own and other groups. In addition, the group of Movassaghi also published an asymmetrically connected dimerization reaction through diazine fragmentation in 2011.[13k] In 2008, de Lera and co-workers accomplished an efficient synthesis of WIN 64821 (**1**) with the method of Movassaghi et al. as a bioinspired dimerization reaction in a seven-step sequence with a fully protected D-tryptophan derivative and expanded it to the preparation of related natural products, including WIN 64745 (**7**), cristatumin C (**8**), and asperdimin (**9**).[9b,10b] More recently, an alternative approach by using a nickel-catalyzed reductive dimerization reaction of C3-brominated pyrrolidinoindoline derivatives to prepare WIN 64821 (**1**) was developed by Oguri and co-workers.[13p] The first total synthesis and structure revision of naseseazine A (**10**) and naseseazine B (**11**) were achieved by Kim and Movassaghi in 2011 by using a Friedel–Crafts-type coupling reaction as a bioinspired heterodimerization reaction.[11b] Reisman and co-workers also reported an elegant and non-biomimetic synthesis of naseseazine A (**10**) and naseseazine B (**11**) that involved a Cu^I-catalyzed arylation reaction of tryptophan derivatives.[13n]

Despite all of these elegant strategies, we were convinced that a more direct bioinspired solution to the preparation of tryptophan-based diketopiperazine alkaloids was possible. In a true biosynthesis, direct dimerization methods of tryptophan without a special protective group on the substrates in aqueous media should be performed. Organic reactions in water are not easy because common organic compounds are usually insoluble in water, and sometimes the water reacts with the substrates or reagents. With alkaloids that contain basic amine portions in the molecule, such situations occur under neutral or basic conditions. In contrast, under acidic conditions, basic alkaloids form water-soluble salts. In addition, salt formation possibly prevents side reactions derived from the nucleophilicity of the lone pair of the amine without any protective groups. Thus, acids would play an important role in aqueous biosynthesis of alkaloids. Recently, Boger and co-workers reported an elegant bioinspired radical-mediated oxidative coupling reaction of vinblastine and related alkaloids in aqueous HCl and 2,2,2-trifluoroethanol.[14]

Our proposed biosynthetic pathway for dimeric diketopiperazine alkaloids is shown in Scheme 1. After the water-soluble salt of tryptophan is formed under acidic conditions, it receives selective one-electron oxidation on the indole core without undesired oxidation of the primary amine. The generated radical compounds have several resonance hybrids, such as active species of the radical on the C3, C7, or N1 atom (A, B, or C in Scheme 1). These intermediates dimerize to provide the corresponding natural product scaffolds: A+A for compounds **1**–**9**, A+B for compounds **10** and **11**, and A+C for compound **12**. Recently, Watanabe and co-workers reported an enzymatic biosynthesis of ditryptophenaline (**4**) through a radical-mediated coupling reaction with cytochrome P450.[15]



Scheme 1. Proposed dimerization reaction of the tryptophan units in the biosynthetic pathway.

In 2013, we reported two one-pot or three-step syntheses of WIN 64821 (**1**), ditryptophenaline (**4**), and naseseazine B (**11**) by using an originally developed, bioinspired dimerization reaction in acidic aqueous media along with our proposed biosynthetic pathway.[16] Full details of the development of the bioinspired dimerization reaction of tryptophan derivatives in aqueous acidic media are provided herein. In addition, total

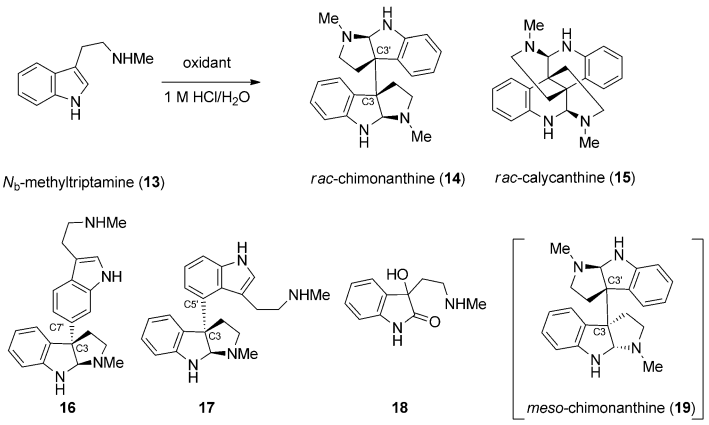
syntheses of WIN 64821 (**1**), eurocristatine (**2**), 15,15′-bis-*epi*-eurocristatine (**3**), ditryptophenaline (**4**), 1′-(2-phenylethylene)-ditryptophenaline (**5**), WIN 64745 (**7**), cristatumin C (**8**), asperdimin (**9**), naseseazine A (**10**), and naseseazine B (**11**), as well as structure determination including the relative stereochemistry of ditryptoleucine A (**6**) by syntheses of four possible candidates by using two one-pot sequences, are also detailed herein. Subsequent extension of the studies detailed herein also provided synthesized natural products and related compounds with an inhibition activity of the ubiquitin-specific protease 7 (USP7) and inhibition activity of foam cell formation in macrophages.

Results and Discussion

Development of a bioinspired dimerization reaction of tryptophan derivatives in water

To achieve the proposed bioinspired dimerization reaction in the flask, N_b-methyltryptamine (**13**) was chosen as the model substrate because the expected dimeric products through a homodimerization reaction at the C3-position were the known natural products *rac*- and *meso*-chimonanthine (Table 1). In addition, syntheses of *rac*- or *meso*-chimonanthine and the naseseazine-type compound **16** by using a hypervalent iodine-mediated dimerization reaction of N_b-methoxycarbonyltryptamine in 2,2,2-trifluoroethanol followed by natrium-bis(2-methoxyethoxy)-aluminum hydride (Red-Al) reduction were reported by Takayama and co-workers.[17] Before starting the screening of oxidants, *rac*- and *meso*-chimonanthine and the heterodimeric compound **16** were prepared by using the protocol of Takayama et al. to obtain authentic samples. Aqueous 1 M HCl solution was determined as the reaction solvent for oxidant screening to provide a homogeneous aqueous solution. Oxidants that promoted oxidative biaryl coupling with phenols or electron-rich aromatics in normal organic solvents were examined.[18] All reactions were carried out in open flasks without special care. $MoCl_5$, $Cu(acac)_2$ (acac = acetylacetonate), $CuBr_2$, and $FeCl_3$ do not promote any reaction as the amount of recovered starting material **13** was above 90% (Table 1, entries 1–4). In addition, $MoCl_5$ immediately decomposed in aqueous media (Table 1, entry 1). On the other hand, after treatment with $Pb(OAc)_4$ or $PhI(OCOCF_3)_2$, the unexpected highly oxidized 3-hydroxyoxindole derivative **18** was obtained in 15 and 32% yield, respectively, as the only isolatable product (Table 1, entries 5 and 6). In contrast, $Mn(OAc)_3 \cdot 2H_2O$ promoted the desired dimerization reaction by maintaining its oxidizing activity in aqueous acidic solution (Table 1, entry 7). Thus, after treatment with 1.5 equivalents of $Mn(OAc)_3 \cdot 2H_2O$, naturally occurring racemic chimonanthine (**14**), which has a C_2-symmetrical C3(sp^3)–C3′(sp^3) bridge, racemic calycanthine (**15**), which was generated from chimonanthine through an acid-mediated rearrangement;[19] the naseseazine-type compound **16**, which has a C3(sp^3)–C7′(sp^2) bridge, and the non-

Table 1. Screening of oxidants for bioinspired oxidative dimerization reactions.[a]



*N*b-methyltriptamine (**13**) *rac*-chimonanthine (**14**) *rac*-calycanthine (**15**)

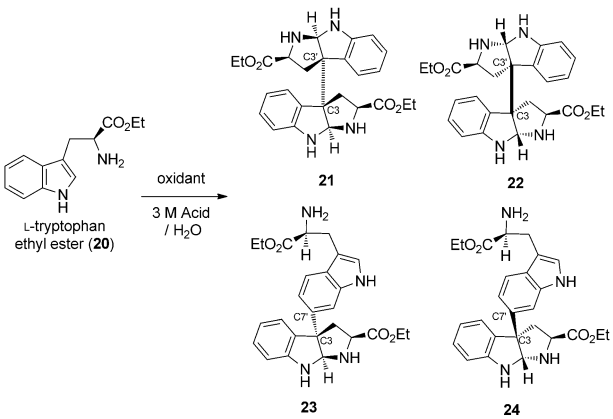
Entry	Oxidant [equiv]	*T* [°C]	*t* [h]	Yield [%][b]					
				14	**15**	**16**	**17**	**18**	recovered **13**
1	$MoCl_5$ (2.0)	23	5	–	–	–	–	–	>90[c]
2	$Cu(acac)_2$ (1.5)	23–80	19	–	–	–	–	–	>90[c]
3	$CuBr_2$ (1.5)	23–80	19	–	–	–	–	–	>90[c]
4	$FeCl_3$ (2.0)	0	19	–	–	-	–	–	>90[c]
5	$Pb(OAc)_4$ (1.4)	0	2	–	–	–	–	15	–
6	$PhI(OCOCF_3)_2$ (1.5)	0	25	–	–	–	–	32	–
7	$Mn(OAc)_3$ (1.5)	0	2	7	1	37	9	–	trace
8	VOF_3 (1.5)	0	2	9	1	30	12	–	12
9	$Ce(SO_4)_2$ (1.5)	0	6	4	trace	22	trace	3	23
10	V_2O_5 (0.55)	0	12	8	trace	50	–	5	19

[a] Reaction conditions: N_b-methyltryptamine (**13**) (0.5 mmol), 1 M HCl/H_2O (3 mL) in an open flask. See the Supporting Information for more details. [b] Yield of the isolated product. [c] Calculated from ^1H NMR spectra of crude material.

symmetrical dimeric compound **17**, which has an unnatural C3(sp^3)–C5′(sp^2) bridge, were isolated in 7, 1, 37, and 9% yield, respectively. The isolated compounds **14** and **15** were identified by comparison with spectral data of natural products and synthesized authentic samples. The structures of compounds **16** and **17**, including their regio- and stereochemistry, were determined by using 2D NMR experiments (i.e., H–H COSY, HMQC, HMBC, and NOESY spectra; see the Supporting Information). By further screening the oxidants, a vanadium oxidant was found to promote the desired dimerization reaction in aqueous acidic media (Table 1, entries 8 and 10). VOF_3 provided a similar result to $Mn(OAc)_3$. In addition, $Ce(SO_4)_2{\cdot}4H_2O$ also promoted the desired dimerization, even though the reactivity was less than for manganese and vanadium oxidants (Table 1, entry 9). In the case of V_2O_5, the yield of compound **16** was increased without production of compound **17** (Table 1, entry 10). At this stage, it was doubted that VOF_3 transforms to V_2O_5 in aqueous acidic media, as described in detail below. On the other hand, *meso*-chimonanthine (**19**) was not observed in any successful reactions despite careful monitoring by using TLC and NMR analysis with an authentic sample.

After discovering that $Mn(OAc)_3$ and VOF_3 were suitable oxidants for our proposed bioinspired dimerization reaction in a flask, commercially available tryptophan ethyl ester (**20**) was chosen as the chiral substrate, because the anticipated dimeric compounds can be quickly accessed as natural products (Table 2). Tryptophan methyl ester could not be applied, because the methoxy carbonyl group is partially hydrolyzed under aqueous acidic conditions. When compound **20** was treated with 1.2 equivalents of $Mn(OAc)_3$ in aqueous 3 M HCl solution, the C_2-symmetrical and non-symmetrical dimeric compounds **21**, **22**, **23**, and **24** were obtained in of 7, 4, 12, and 9% yield, respectively, along with recovered starting material **20** (Table 2, entry 1). All isomers were easily separated by double normal-phase silica gel column chromatography (see the Supporting Information for full details). After confirming that the desired reaction could be applied to tryptophan derivatives, the acid effects were screened for optimization. When aqueous HCl, H_2SO_4, CH_3SO_3H, CF_3CO_2H, Cl_3CCO_2H, and H_3PO_4 ($pK_a = -8.0$ to 2.12 in H_2O) were employed as reaction media, the desired dimerization reaction proceeded effectively (Table 2, entries 2–6). In particular, CH_3SO_3H ($pK_a = -2.6$ in H_2O) provided the best conversion. Thus, the dimeric compounds **21**, **22**, **23**, and **24** were obtained in 19, 11, 28, and 21% yield, respectively, and the total yield of dimeric compounds was 79% (Table 2, entry 3). In contrast, weaker acids such as HCO_2H ($pK_a = 3.77$ in H_2O) and CH_3CO_2H ($pK_a = 4.76$ in H_2O) did not produce any products at 0 °C to ambient temperature, even though the substrate was cleanly dissolved in the aqueous solution. This result indicates that the role of the acid is not only to promote salt formation of the amine portion to dissolve it in H_2O and protection of the lone pair of the amine, but also activation of the metal oxidant. For VOF_3 as oxidant, CH_3SO_3H was also a suitable acid to provide almost the same result as $Mn(OAc)_3$ (see Table 2, entry 9, and full details of the acid effect by using VOF_3 and V_2O_5 in the Supporting Information).

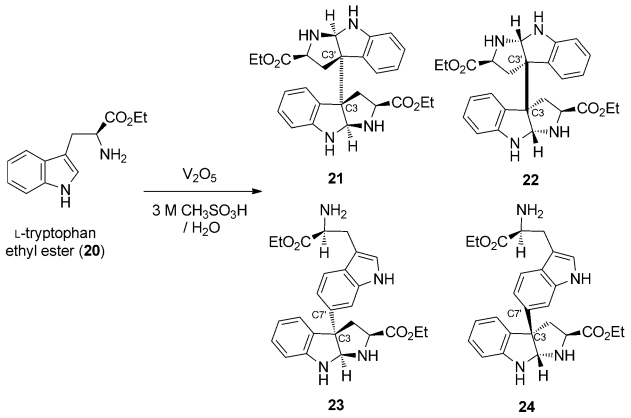
Table 2. The acid effect in bioinspired oxidative dimerization reactions with tryptophan ethyl ester.[a]



Entry	Oxidant [equiv]	Acid [pKa]	T [°C]	t [h]	Yield [%][b] 21	22	23	24	recovered 20
1	$Mn(OAc)_3$ (1.5)	HCl (−8.0)	0	0.7	7	4	12	9	37
2		H_2SO_4 (−3.0)	0	0.7	15	8	23	20	9
3		CH_3SO_3H (−2.6)	0	0.7	19	11	28	21	4
4		CF_3CO_2H (−0.25)	0	2	11	10	25	19	14
5		CCl_3-CO_2H (0.65)	0	2	12	9	27	20	14
6		H_3PO_4 (2.12)	0	2	12	8	21	17	12
7		HCO_2H (3.77)	0–23	24	–	–	–	–	>90[c]
8		AcOH (4.76)	0–23	24	–	–	–	–	>90[c]
9	VOF_3 (1.5)	CH_3SO_3H (−2.6)	−10	0.5	19	11	28	24	8

[a] Reaction conditions: L-tryptophan ethyl ester (**20**) (0.5 mmol), 3 M acid/H_2O (3 mL) in an open flask. See the Supporting Information for more details. [b] Yield of the isolated product. [c] Calculated from ^1H NMR spectra of crude material.

Table 3. The bioinspired oxidative dimerization reactions with V_2O_5 as oxidant.[a]



Entry	V2O5 [equiv]	T [°C]	t [h]	Initial state of the reaction	Yield [%][b]				
					21	**22**	**23**	**24**	recovered **20**
1[c]	0.55	0	0.7	heterogeneous	trace	trace	trace	trace	>90[d]
2[e]	0.55	−10	6	homogeneous	16	9	26	18	11
3	0.55	−10	45	heterogeneous	21	25	16	13	4
4	0.65	−15	129	heterogeneous	28	28	15	10	0
5[f]	0.65	−15	192	heterogeneous	26	27	9	10	0

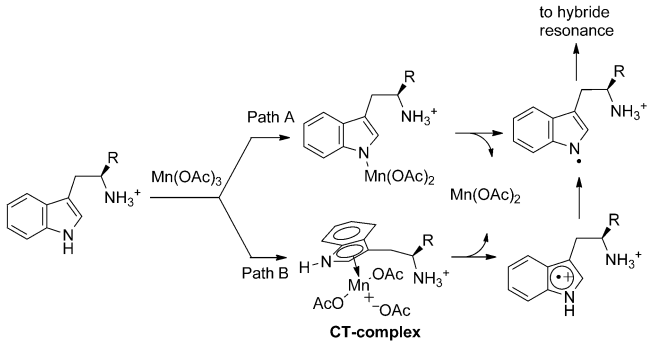
[a] Reaction conditions: L-tryptophan ethyl ester (**20**) (0.5 mmol), 3 M acid/H_2O (10 mL) in an open flask. See the Supporting Information for more details. [b] Yield of the isolated product. [c] Six equivalents of HF were added to the reaction mixture as additive. [d] Calculated from ^{1}H NMR spectra of crude material. [e] Before addition of L-tryptophan ethyl ester (**20**), V_2O_5 was stirred for 40 min in 3 M $MeSO_3H$ at 23 °C to provide a homogeneous solution. [f] L-Tryptophan ethyl ester (**20**) (6.0 g, 25.8 mmol), 3 M $MeSO_3H$ in H_2O (517 mL) in an open flask. See the Supporting Information for details.

After screening of the acid effect with VOF_3 as oxidant, we considered the possibility of hydrolysis of VOF_3 to generate V_2O_5 [Eq. (1)]. Thus, V_2O_5 was selected as another oxidant for the dimerization reaction (Table 3). When compound **20** was treated with 0.55 equivalents of V_2O_5 in the presence of 6 equivalents of HF under the same reaction conditions as when using VOF_3 as the oxidant (Table 2, entry 9), the desired dimerization reaction did not proceed well and dimeric products were just detected in trace amounts (Table 3, entry 1). In addition, V_2O_5 was not dissolved and the state of the reaction was heterogeneous. (In contrast, the reaction with VOF_3 was homogeneous.) Therefore, before the addition of compound **20** to start the oxidation reaction, the mixture of V_2O_5 in aqueous CH_3SO_3H solution was stirred for 40 min at 23 °C to generate a homogeneous solution (Table 3, entry 2). As a result, the reaction was completed after 6 h and provided almost the same result as VOF_3 (Table 2, entry 9). This result indicates that vanadium(V) methanesulfonate oxide complexes such as $VO_2(CH_3SO_3)$ and $VO(CH_3SO_3)_3$ might be generated in situ from both VOF_3 and V_2O_5, and act as the actual active reagent in the reaction systems.

$$2\,VOF_3 + 3\,H_2O \rightarrow V_2O_5 + 6\,HF \qquad (1)$$

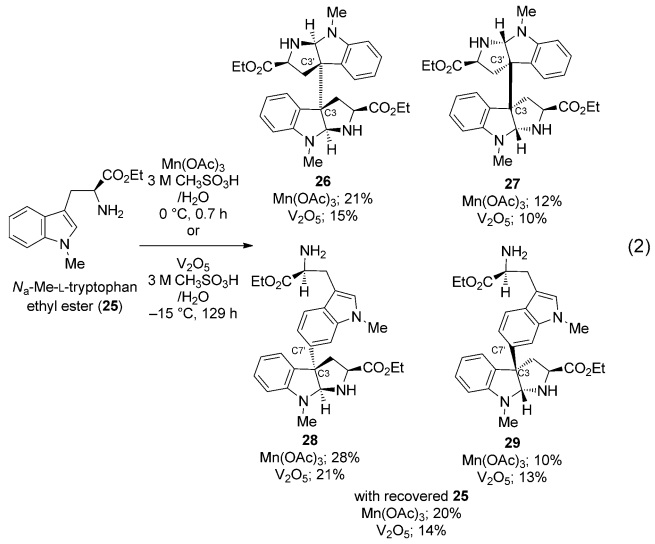
On the other hand, V_2O_5 under heterogeneous conditions at the initial stage of the reaction in aqueous CH_3SO_3H was the most effective reaction oxidant to form the C_2-symmetrical compounds, even though longer reaction times were needed to complete the reaction (Table 3, entry 3). Thus, when compound **20** was treated with 0.55 equivalents of V_2O_5 at −10 °C for 45 h, compounds **21** and **22** were obtained in 21 and 25% yields, respectively. In this reaction, V_2O_5 was slowly dissolved in the aqueous CH_3SO_3H solution, and then a homogeneous solution was generated at the end of the reaction. After slight optimization of the amount of equivalents and the reaction temperature, the selectivity of the dimeric compounds was improved to provide both compounds **21** and **22** in 28% yields (Table 3, entry 4, 0.65 equiv of V_2O_5, −15 °C, 129 h). In addition, the conversion was superb; the total yield of the dimeric compounds was 80%. Furthermore, the dimerization reaction supplied the desired dimeric compounds in multigram amounts by a one-step procedure (Table 3, entry 4). Under heterogeneous conditions, the ratio of the C_2-symmetrical compounds was increased to compare with homogeneous reaction conditions by using V_2O_5 (Table 3, entry 2 vs. entry 3). The C_2-symmetrical oxidative dimerization might profitably proceed on the surface of solid V_2O_5.

The mechanism for producing the key radical species on the indole core by using metal oxidants was considered to proceed through two possible pathways (Scheme 2). One of the proposed pathways is the oxidation reaction by forming an indole nitrogen–metal covalent bond followed by a single-electron transfer (Scheme 2, Path A).[20] Another possibility was the direct oxidation of an indole π-bond through a charge-transfer (CT) complex followed by a single-electron transfer and deprotonation (Scheme 2, Path B).[18g,21] To determine the reaction mechanism, we investigated the dimerization reaction by using N_a-Me tryptophan ethyl ester (**25**), because the sub-



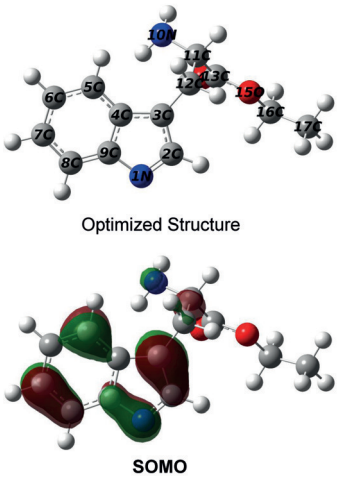
Scheme 2. Proposed reaction mechanisms.

strate should not undergo the oxidation reaction through path A [Eq. (2)]. As a result, the dimerization reactions with $Mn(OAc)_3$ under homogeneous condition (see Table 2, entry 3) and with V_2O_5 under heterogeneous condition (see Table 3, entry 4) proceeded smoothly to provide four dimeric compounds, which showed similar productivity as in the case of tryptophan ethyl ester (**20**). These results supported the pathway through a CT complex for generation of the key radical intermediate.



(2)

On the other hand, all obtained dimeric compounds possessed a C3 quaternary carbon atom. This result indicated that the C3 radical widely existed as the most stable intermediate among the resonance hybrids. Therefore, we investigated the effect of radical stability on the indole core of tryptophan ethyl ester (**20**) by using theoretical calculations (Figure 2, calculation method: B3PW91/6-311+G(d, p), Gaussian 09 Rev.C01).[22] As a result, the singly occupied molecular orbital (SOMO) on the C3 carbon atom was observed to be larger than the orbitals on the other carbon atoms. In addition, the Mulliken spin density of the C3 carbon atom showed a large positive value. Both calculation results strongly supported that the C3 radical intermediate was the most stable form in the bioinspired dimerization reactions.



Mulliken atomic spin densities

No.	atom	spin densities
1	N	0.279
2	C	-0.131
3	C	0.664
4	C	-0.183
5	C	0.261
6	C	-0.070
7	C	0.173
8	C	-0.020
9	C	0.040
10	N	0.006
11	C	-0.057
12	C	-0.005
13	C	0.039
14	O	0.000
15	O	0.001
16	C	0.001
17	C	-0.001

Figure 2. SOMO of tryptophan ethyl ester (**20**) (method: B3PW91/6-311+G(d,p), Gaussian09 Rev.C01)[22].

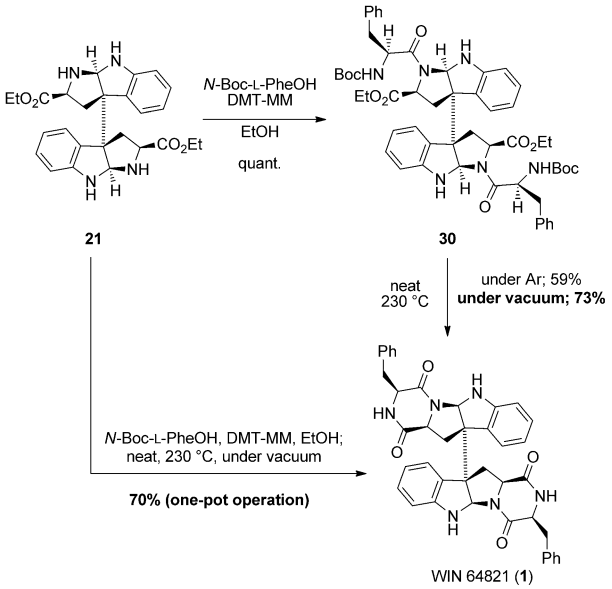
With large amounts of these key dimeric intermediates for the bioinspired synthesis in hand, our focus moved to the short-step syntheses of tryptophan-based dimeric diketopiperazine alkaloids.

Synthesis of WIN 64821, eurocristatine, and 15,15′-bis-*epi*-eurocristatine

WIN 64821 (**1**, also called Q20547A), which contains a C3(sp^3)–C3′(sp^3) bridge and two tryptophan and phenylalanine units, was independently isolated from *Aspergillus* species by using bioactivity guided investigations by the groups of Barrow (1993)[2a] and Hiramoto (1994).[2e] It is reported to be a competitive substance P antagonist with respect to the human neurokinin-1 and the cholecystokinin B receptor, and also an antagonist of the cholecystokinin type-B receptor. The absolute stereochemistry of compound **1** was estimated from its circular dichroism spectrum. Furthermore, structure–activity relationship studies of compound **1** by using biosynthetic analogues have also been reported.[2] Several total syntheses of compound **1** were reported.[10b, 13] Eurocristatine (**2**, also called cristatumin E), which contains two L-tryptophan and D-valine units, was independently isolated from *Eurotium cristatum* by the group of Kijjoa (2012)[5] and from *Eurotium herbariorum* by the group of Zhu (2013).[3] The absolute stereochemistry was determined by Marfey's amino acid analysis for the valine unit and estimated by a CD spectrum for the C3 atom of the tryptophan unit, followed by confirmation by X-ray analysis. In addition, it is reported to have a weak antibacterial activity and cytotoxic activity. Eurocristatine (**2**) was synthesized as an undesired side product in the total synthesis of cristatumin C (**8**) by the group of de Lera in 2014; they might not have recognized it as a natural product.[9b] 15,15′-bis-*epi*-Eurocristatine (**3**), which contains two L-tryptophan and L-valine units, was prepared by using an enzymatic feeding experiment for the prep-

aration of an analogue of compound **1** and it is reported to have moderate activity as a cholecystokinin type-B receptor antagonist.[2e] The enantiomer of compound **3** was synthesized by de Lera and co-workers for an analytical study of the configuration and conformation of this type of diketopiperazine alkaloid by using NMR and ECD spectroscopy supported by DFT calculations.[23] The naturally occurring compounds **1**, **2**, and **3** are C_2-symmetrical compounds that contain a dimeric tryptophan unit possessing a C3(*R*)–C2(*R*) configuration, and the same two amino acids, L-phenylalanine, D-, or L-valine. Thus, the synthesis of compounds **1**, **2**, and **3** could be accomplished by a condensation reaction of the dimeric intermediate **21** and the corresponding two amino acids followed by diketopiperazine formation. The group of de Lera had already established an excellent procedure of a condensation reaction with the dimeric compound prepared by four steps and 9-fluorenylmethoxycarbonyl (Fmoc)-protected amino acids by using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*,*N*-tetramethyluronium hexafluorophosphate (HATU) as the coupling reagent.[10b,13d]

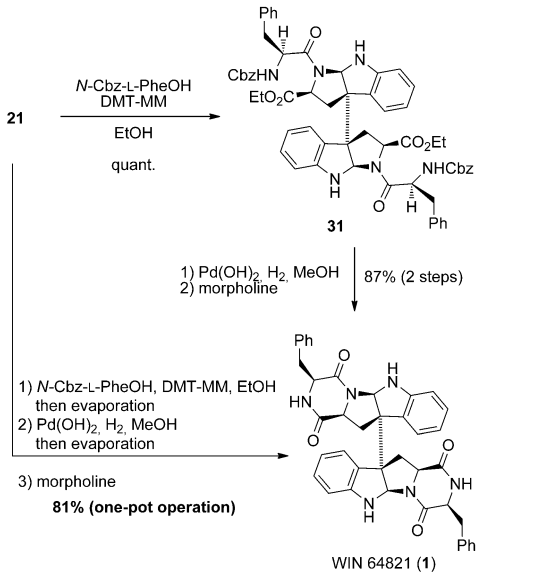
On the other hand, the one-pot reaction is one of the key topics in chemistry, underlining the importance of the "Pot economy."[24] A one-pot reaction is an effective method to conduct several transformations in a single reaction vessel along with cutting out several purifications, minimizing chemical-waste generation, and saving time. Therefore, we decided to adopt a one-pot reaction for a simple, concise, and effective synthesis of the title compounds. At first, for the dehydration condensation reaction to apply in a one-pot operation, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM), developed by Kunishima and co-workers,[25] was employed because the reagent can be used in alcohol as solvent, which is environmentally friendly, without any additives such as bases. For the synthesis of compound **1**, treatment of compound **21** in EtOH with 2.1 equivalents of *N*-Boc-phenylalanine (Boc = *tert*-butyloxycarbonyl) in the presence of 2.2 equivalents of DMT-MM (0 °C, 4 h) in an open flask cleanly provided compound **30** in superb conversion (quantitative yield, Scheme 3). Initial attempts to convert compound **30** directly to compound **1** including removing of the Boc group and intramolecular amide formation to construct the diketopiperazine ring in a single step, provided compound **1** in 59% yield under an argon atmosphere at 230 °C in the absence of solvent. The major side products were estimated as epimerized compounds at the α-position of the amide carbonyl group by NMR analysis. After careful monitoring of the reaction, we noticed that a trace amount of produced EtOH was present at the bottom of the reaction vessel, even though the temperature of the oil bath was 230 °C. We expected that this polar solvent induced the epimerization reaction at high temperature. Therefore, the diketopiperazine formation reaction was carried out under vacuum conditions (0.1 mbar) so that the produced EtOH was immediately removed from the reaction vessel. As a result, the yield of compound **1** was improved to 73% and epimerized side products were obviously decreased, as shown by analysis of the ^{1}H NMR spectra of the crude material. Finally, a condensation reaction and diketopiperazine formation could be conducted simultaneously as a one-pot operation. Thus, after the



Scheme 3. Synthesis of WIN 64821 (**1**) through a one-pot procedure with Boc-L-PheOH.

condensation reaction of the dimeric compound **21** with *N*-Boc-L-phenylalanine (2 equiv) in the presence of DMT-MM (2 equiv, EtOH, 0 °C, 4 h) was complete, the solvent was removed under reduced pressure at ambient temperature. The reaction flask under vacuum conditions (0.1 mbar, 25 min) was directly put into an oil bath at 230 °C to provide compound **1** in superb yield (70%).

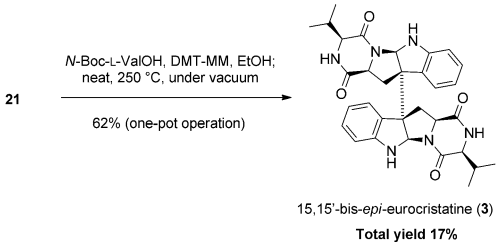
An alternative one-pot procedure for diketopiperazine formation in the presence of solvent was developed because some solid precursors with high melting points could not be applied under neat conditions (Scheme 4). Thus, treatment of the resolved dimeric compound **21** (1 equiv) and *N*-Cbz-L-phe-



Scheme 4. Synthesis of WIN 64821 (**1**) through a one-pot procedure with Cbz-L-PheOH.

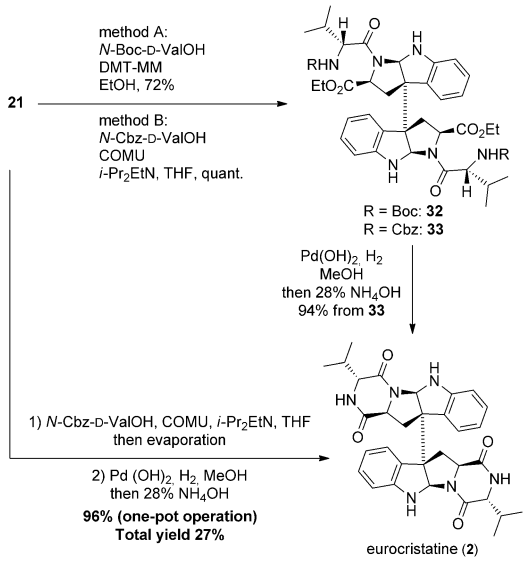
nylalanine (2.1 equiv) (Cbz = carbobenzyloxy) in the presence of 2.2 equivalents of DMT-MM cleanly provided the bisamide **31** (EtOH, 0 °C, 8 h, quantitative yield). Removal of the Cbz group and diketopiperazine formation was accomplished by hydrogenation followed by treatment with morpholine[13r] for the cyclization reaction in superb yield (87%). After establishing a separate protocol, a one-pot transformation was examined. When the coupling reaction of compound **21** and the amino acid was completed, EtOH was removed under reduced pressure. To the crude mixture in MeOH, $Pd(OH)_2$ was added and the reaction mixture was stirred under hydrogen. The solvent swapping from EtOH to MeOH was important because the produced primary amine intermediates were insoluble in EtOH. After completion of hydrogenation, removal of the solvent followed by addition of morpholine provided WIN 64821 (**1**) in superb yield (81%). The total yield of compound **1** from compound **20** was 23% by using two one-pot sequences including the V_2O_5 oxidation reaction and diketopiperazine formation.

Total synthesis of 15,15′-bis-*epi*-eurocristatine (**3**) was accomplished by a one-pot procedure with *N*-Boc-L-valine [Eq. (3)]. Thus, after treatment of compound **21** with 2.1 equivalents of *N*-Boc-L-valine and DMT-MM (2.2 equiv, EtOH, 0 °C, 10 h) led to a bisamide intermediate, the solvent was removed under reduced pressure followed by heating at 250 °C while maintaining vacuum conditions (0.1 mbar, 30 min) to provide compound **3** in 62% yield. The total yield of compound **3** from compound **20** was 17% by using two one-pot sequences.



(3)

To achieve the synthesis of eurocristatine (**2**), *N*-Boc-D-ValOH (2.1 equiv) was added to the dimeric compound **21** with DMT-MM (2.1 equiv) to afford the bisamide compound **32** (EtOH, 0 °C, 10 h, 72%, Scheme 5, method A). The yield was not sufficient for application to a one-pot procedure. Therefore, several coupling reagents and conditions were rescreened. As a result, (1-cyano-2-ethoxy-2-oxoethylideneaminooxy)dimethylaminomorpholinocarbenium hexafluorophosphate (COMU) was found to be an effective coupling reagent (Scheme 5, method B).[26] Thus, treatment of *N*-Cbz-D-ValOH (2.1 equiv) with COMU (2.2 equiv) in the presence of iPr_2EtN (4.0 equiv, THF, 0 °C, 12 h) provided the desired bisamide compound **33** in quantitative yield. Removal of the Cbz group and diketopiperazine formation was accomplished by a hydrogenation reaction followed by treatment with an aqueous ammonia solution to provide eurocristatine in superb yield

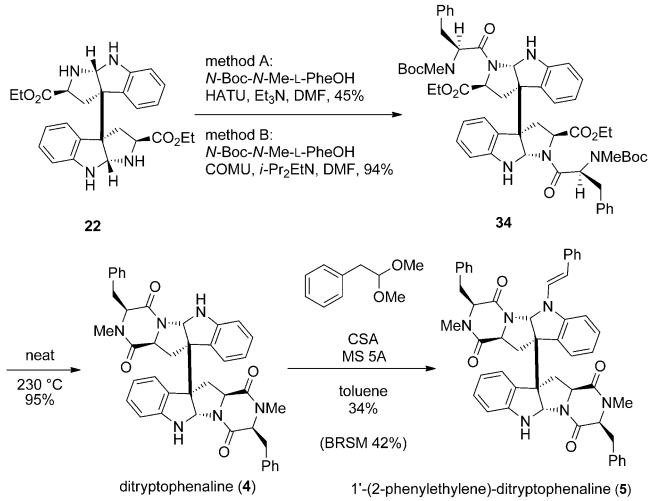


Scheme 5. Synthesis of eurocristatine (**2**).

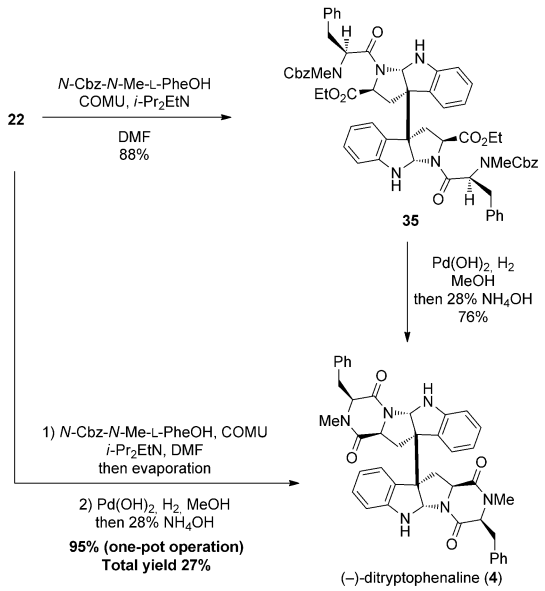
(94%). Then, we conducted the separate procedure as a one-pot sequence. After the coupling reaction with COMU and *N*-Cbz-D-Val-OH, excess iPr_2EtN and THF were removed under vacuum. The obtained crude mixture was treated with $Pd(OH)_2$ under hydrogen gas in MeOH followed by the addition of 28% aqueous ammonia solution to afford compound **2** in 96% yield. Total yield of compound **2** from compound **20** was 27% by using two one-pot sequences.

Synthesis of ditryptophenaline and 1′-(2-phenylethylene)-ditryptophenaline

(−)-Ditryptophenaline (**4**) was isolated from *Aspergillus flavus* and reported to be a weak competitive substance P antagonist with respect to human neurokinin-1 and the cholecystokinin B receptor.[2,6] In 1994, 1′-(2-phenylethylene)-ditryptophenaline (**5**) was isolated from the same *Aspergillus* species as the non-symmetrical ditryptophenaline analogue.[7] The stereochemistry of compounds **4** and **5** is a C3(*S*)–C2(*S*) configuration, and two *N*-methyl-L-phenylalanines were involved in the dimeric moiety. The synthetic study of ditryptophenaline (**4**) started from the condensation reaction with *N*-Boc-*N*-methyl-L-phenylalanine. Surprisingly, when the coupling reaction with DMT-MM was applied to compound **22** with *N*-Boc-*N*-methyl-L-phenylalanine, the starting material **22** was completely recovered. In addition, *N*-methyl-L-phenylalanine ethyl ester was obtained as the only isolable product. Therefore, other coupling reagents and conditions were widely screened. After several attempts to convert compound **22** to compound **34**, we found that HATU (2.4 equiv) and Et_3N (5.9 equiv) gave the desired coupling reaction in moderate yield (45%, Scheme 6, method A). Finally, COMU was the best reagent as the coupling reagent to synthesize compound **4**. Thus, the dehydration condensation reaction with compound **22** (1 equiv) and *N*-Boc-*N*-Me-L-phenylalanine (2.4 equiv) in the presence of COMU



Scheme 6. Synthesis of ditryptophenaline (**4**) and 1′-(2-phenylethylene)-ditryptophenaline (**5**) with *N*-Boc-*N*-Me-L-PheOH.



Scheme 7. Synthesis of ditryptophenaline (**4**) with *N*-Cbz-*N*-Me-L-PheOH through a one-pot sequence.

(2.4 equiv, DMF, 0 °C, 60 h) provided compound **34** in superb yield (94%, Scheme 6, method B). Subsequent diketopiperazine construction to ditryptophenaline (**4**) was accomplished by heating at 230 °C under neat conditions in excellent yield (95%). Notably, the epimerization reaction of the carbonyl group of bisamide **34** was not observed under the neat conditions. In addition, this three-step total synthesis of compound **4** was accomplished on a multigram scale; 1.2 g of compound **4** were obtained in one sequence. Transformation from compound **4** to 1′-(2-phenylethylene)-ditryptophenaline (**5**) was accomplished by using a modified Movassaghi protocol.[13e] Thus, treatment of compound **4** with (2,2-dimethoxyethyl)benzene (1 equiv) in the presence of camphor sulfonic acid (CSA, 1 equiv) and MS 5 A (toluene, 23 °C, 22 h) afforded the nonsymmetric compound **5** in moderate yield [34%, with C_2-symmetric 1, 1′-bis(2-phenylethylene)ditryptophenaline (29% based on recovered starting material) 42%; for more details see the Supporting Information].

Even though an efficient total synthesis of compound **4** was achieved, these separate reaction conditions could not be conducted as a one-pot protocol because the thermal neat conditions with the destruction of COMU or HATU provided a complex mixture. For a one-pot operation from compound **22** to compound **4**, *N*-Cbz-*N*-methyl-L-phenylalanine was chosen as the coupling partner because the Cbz group could be removed by hydrogenation under mild reaction conditions (Scheme 7). Treatment of the dimeric compound **22** with *N*-Cbz-*N*-methyl-L-phenylalanine (2.4 equiv) in the presence of COMU (2.4 equiv, iPr$_2$EtN, DMF, 0 °C, 40 h) provided the coupling product **35** in excellent yield (88%). Subsequent diketopiperazine formation to provide compound **4** was achieved by a hydrogenation reaction ($Pd(OH)_2$, H_2, MeOH) followed by addition of aqueous ammonia solution in 76% yield. Finally, these two steps combined to a one-pot operation. Thus, when the coupling reaction with compound **22** and *N*-Cbz-*N*-methyl-L-phenylalanine was complete, the solvent and excess iPr$_2$EtN were removed from the reaction vessel. Treatment of the resolved bisamide **35** with $Pd(OH)_2$ under a hydrogen atmosphere in MeOH and subsequent base treatment cleanly provided compound **4** in superb yield (95%). The total yield of compound **4** from compound **20** was 27% through two one-pot sequences.

Synthesis and structure determination of ditryptoleucine A

In 2012, ditryptoleucine A and ditryptoleucine B were isolated from *A. oryzae* as a diastereomeric mixture.[8] Their planar structures were determined by NMR spectroscopy and mass spectrometry, but stereochemical information was not reported. These structures contained two units of *N*-methyl-L-leucine, which was incorporated into a tryptophan-based dimeric structure. In addition, a C_2-symmetric structure was suggested by NMR analysis. On the basis of the above-mentioned information, the structures including the relative stereochemistry of ditryptoleucine A and ditryptoleucine B were narrowed down to five (Figure 3). On the other hand, previously reported tryptophan-based dimeric diketopiperazine alkaloids consisted of two L-tryptophan units. A dimeric *meso* compound that contained L- and D-tryptophan units in the molecule was not reported. Therefore, we judged it was unlikely to be the *meso* compound **39**. To determine the exact structure of ditryptoleucine A and ditryptoleucine B, the possible candidates **36**, **37**, **38**, and **6** were synthesized by using our developed two one-pot strategies.

Compound **36**, which contains a C3(*R*)–C15(*S*) configuration, was synthesized by using the coupling reaction of the dimeric compound **21** and *N*-Boc-*N*-Me-L-leucine (2.1 equiv) in the presence of DMT-MM (2.2 equiv, EtOH, 0 °C, 10 h) followed by diketopiperazine formation at 250 °C under neat conditions (29%, unoptimized condition, full details of a separate two-step procedure are described in the Supporting Information;

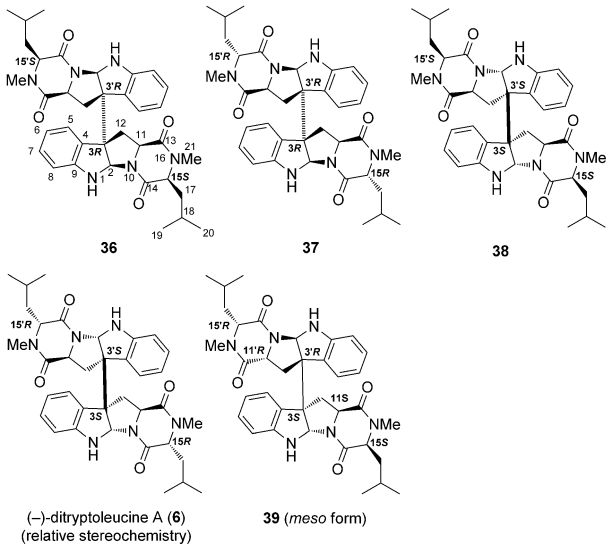
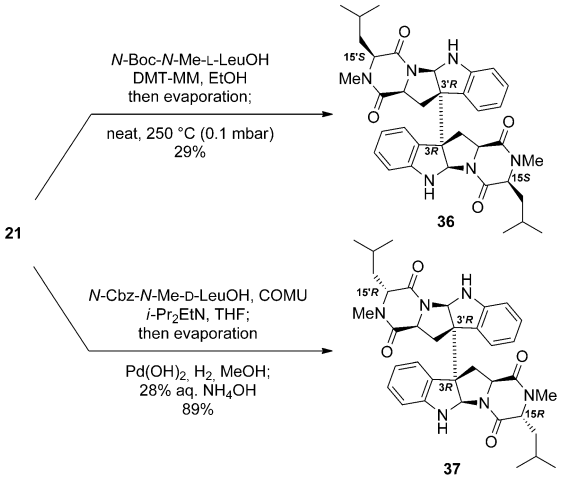
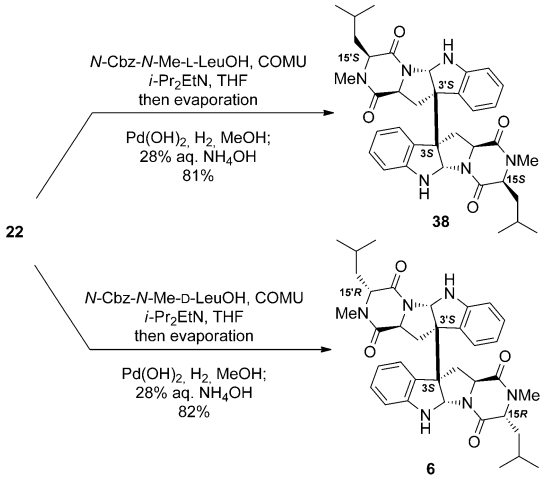


Figure 3. Candidates for ditryptoleucine A and ditryptoleucine B.



Scheme 8. Synthesis of compounds **36** and **37**.



Scheme 9. Synthesis of compound **38** and ditryptoleucine A (**6**).

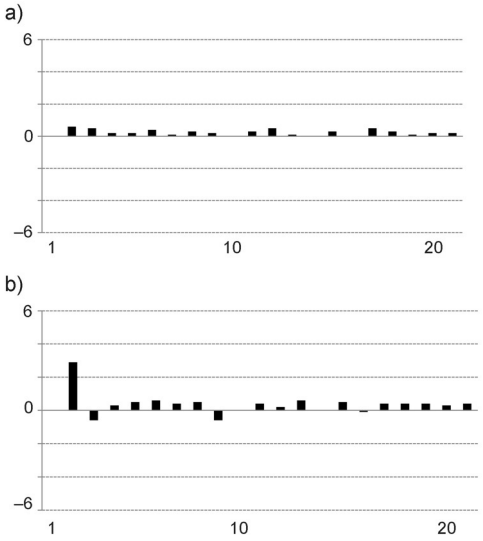


Figure 4. Difference between carbon chemical shifts of a) ditryptoleucine A and **6**, and b) ditryptoleucine B and **37**.

Scheme 8). The one-pot procedure with COMU as coupling reagent with D-leucine derivatives was applied to the synthesis of compound **37**, which contains a C3(*R*)–C15(*R*) configuration. Thus, treatment of compound **21** with *N*-Cbz-*N*-Me-D-leucine (2.1 equiv) in the presence of COMU (2.2 equiv, iPr$_2$EtN, THF, 0 °C, 12 h) provided the bisamide intermediate. After removing the excess amount of base and solvent, treatment of the resolved crude material with $Pd(OH)_2$ under hydrogen gas followed by addition of 28% NH_4OH for diketopiperazine formation provided the desired compound **37** in 89% yield.

The same strategy as for the synthesis of compound **37** was applied for the synthesis of compounds **38** and **6** from the C3(*S*)-dimeric compound **22** (Scheme 9). Thus, compound **38**, which contains a C3(*S*)–C15(*S*) configuration, and compound **6**, which contains a C3(*S*)–C15(*R*) configuration, were prepared in 81 and 82% yields, respectively. After completing the total syntheses of the four candidates of ditryptoleucine A and ditryptoleucine B, a careful comparison of the NMR data with the natural product was carried out (Figure 4). As a result, compound **6** was well identified to be ditryptoleucine A (Figure 4-1; see the Supporting Information for a full comparison of the ^{1}H and ^{13}C NMR spectra of the synthetic compounds and ditryptoleucine A). Thus, we determined the correct structure of ditryptoleucine A, including the relative stereochemistry. Unfortunately, additional information of the chirality of the natural products was not obtained. However, to the best of our knowledge, all reported tryptophan-based dimeric diketopiperazine alkaloids from nature are derived from L-tryptophan, without exception. Therefore, compound **6** is more likely to be a true natural product. On the other hand, the structure of ditryptoleucine B, which is a minor component of naturally occurring diastereomer mixtures, is still unclear. The ^{13}C NMR spectrum of compound **37** was in good agreement with ditryptoleucine B except for the C2 carbon atom (Figure 4-2). The C2 carbon

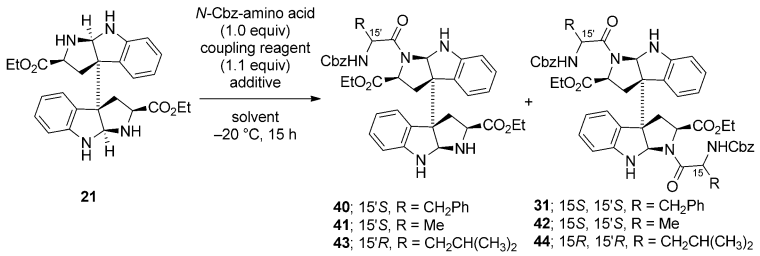
atom belongs to the aminal group, and therefore, salt formation was doubted. Thus, several acid treatments were examined by NMR studies. However, the N1 nitrogen atom, which also belonged to aniline, was quite stable to acid. As a result, no significant differences of the ^{1}H and ^{13}C NMR spectra were observed. Unfortunately, we were not able to get a direct comparison of the NMR charts of the synthetic compound and a natural ditryptoleucine B mixture. It might be the *meso* dimeric compound **39** or simply a mistake in the reported literature.

Synthesis of WIN 64745, cristatumin C, and asperdimin

In nature, heterodimeric diketopiperazine alkaloids that condense with two different amino acids to a C_2-symmetric tryptophan dimer are produced. For instance, WIN 64745 (**7**) was isolated from *Aspergillus* species along with isolation of WIN 64821 (**1**).[2a] The structure was constructed from a C_2-symmetric tryptophan dimer, L-phenylalanine, and L-leucine. Cristatumin C (**8**), which consists of two tryptophan, L-alanine, and D-valine units was isolated from *Eurotium cristatum* EN-220 in 2012.[9a] Asperdimin (**9**), which consists of two tryptophan, D-leucine, and D-valine units was isolated from extracts of *A. niger*, and antiviral activity against certain viruses was reported in 2004.[10a] The reported structures of compounds **8** and **9** at the isolation and structure determination stages were revised by total synthesis.[9b,10b] The excellent pioneering total syntheses of these alkaloids to revise or confirm the exact structure were reported by de Lera and co-workers.[9b,10b,13d] In their total syntheses, stepwise condensation reactions with two different amino acids with HATU as coupling reagent followed by diketopiperazine formation were employed. Our purpose was the accomplishment of two one-pot syntheses of these heterodimeric diketopiperazine alkaloids. Therefore, the screening of monoamidation with the corresponding amino acid to the dimeric intermediate **21** by using DMT-MM or COMU was examined (Table 4). For the synthesis of WIN 64745 (**7**), one equivalent of *N*-Cbz-L-phenylalanine was added to the dimeric intermediate **21** by using DMT-MM (1.1 equiv) as coupling reagent in EtOH at −20 °C to provide the desired monoamide compound **40** in moderate yield (52%) with the undesired bisamide compound **31** (16%, Table 4, entry 1). When COMU was employed as the coupling reagent in the presence of iPr$_2$EtN in THF, the yield of the desired monoamide **40** was improved to 72% with satisfying selectivity (Table 4, entry 2). In addition, the COMU procedure could be applied to the coupling reaction with *N*-Cbz-L-alanine and *N*-Cbz-D-leucine for the preparation of cristatumin C (**8**) and asperdimin (**9**), respectively (Table 4, entries 3 and 4). Even though the yield of the coupling reaction with *N*-Cbz-D-leucine was moderate, the crucial problem was the stability of the monoamide **43** during silica gel chromatography for purification. Therefore, improvement of the total yield was attempted in a one-pot protocol without purification at the monoamide stage.

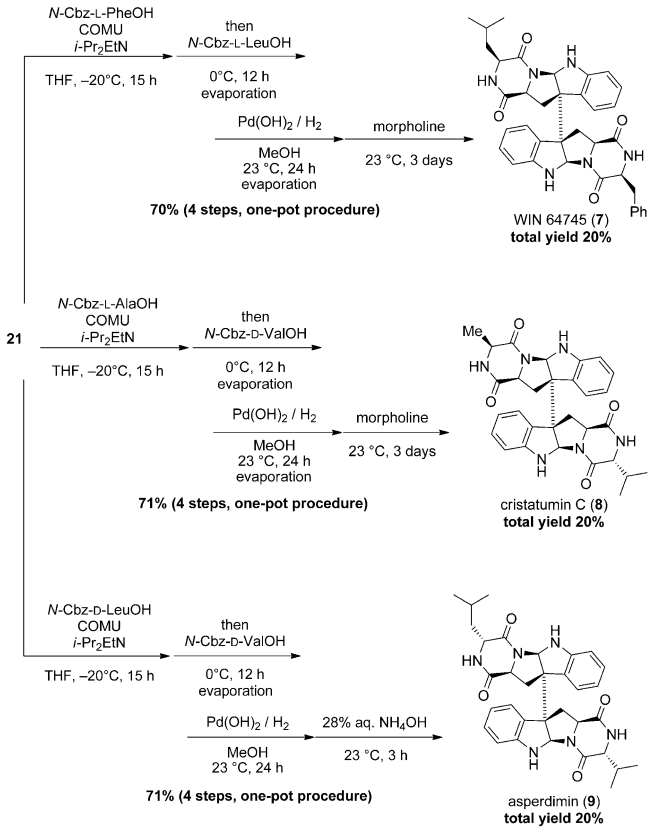
After establishment of the monoamidation, a one-pot transformation from the dimeric compound **21** to the heterodimeric natural products was performed (Scheme 10). Thus, compound **21** was treated with one equivalent of *N*-Cbz-L-phenylalanine in the presence of 2.2 equivalents of COMU and iPr$_2$EtN (4 equiv, THF, −20 °C, 15 h) to provide the monoamide product for the synthesis of WIN 64745 (**7**). The second amino acid, which was *N*-Cbz-L-leucine (1.1 equiv), was added to the reaction mixture to complete the second amidation reaction (0 °C, 12 h). After removing the solvent, a hydrogenation reaction for the deprotection of the Cbz group was carried out by using $Pd(OH)_2$ under a hydrogen atmosphere in the same flask. Finally, treatment with morpholine for diketopiperazine formation provided WIN 64745 (**7**) in superb yield (70%) in a one-pot procedure including a four-step sequence. The total yield of compound **7** from tryptophan ethyl ester (**20**) was 20%. The same protocol was applied to the synthesis of cristatumin C (**8**) with *N*-Cbz-L-alanine and *N*-Cbz-D-valine as coupling partners. Cristatumin C (**8**) was obtained in 71% yield in a one-pot procedure and the total yield was 20%. For the synthesis of asper-

Table 4. Monoamidation reactions for the synthesis of heterodimeric diketopiperazine alkaloids[a]



Entry	Amino acid	Coupling reagent	Additive [equiv]	Solvent	Results[c]	
1[b]	L-Phe-OH	DMT-MM	–	EtOH	**40** (52%)	**31** (16%)
2	L-Phe-OH	COMU	iPr$_2$EtN (3.0)	THF	**40** (72%)	**31** (13%)
3	L-Ala-OH	COMU	iPr$_2$EtN (3.0)	THF	**41** (73%)	**42** (11%)
4	D-Leu-OH	COMU	iPr$_2$EtN (3.0)	THF	**43** (58%)	**44** (10%)

[a] Reaction conditions: compound **21** (0.11 mmol), amino acid (0.11 mmol), coupling reagent (1.2 mmol), and additive in solvent (1.5 mL) in an open flask. The reaction mixture was stirred for 15 h at −20 °C. See the Supporting Information for details. [b] The reaction mixture was stirred for 24 h at −20 °C. [c] Yield of the isolated product.

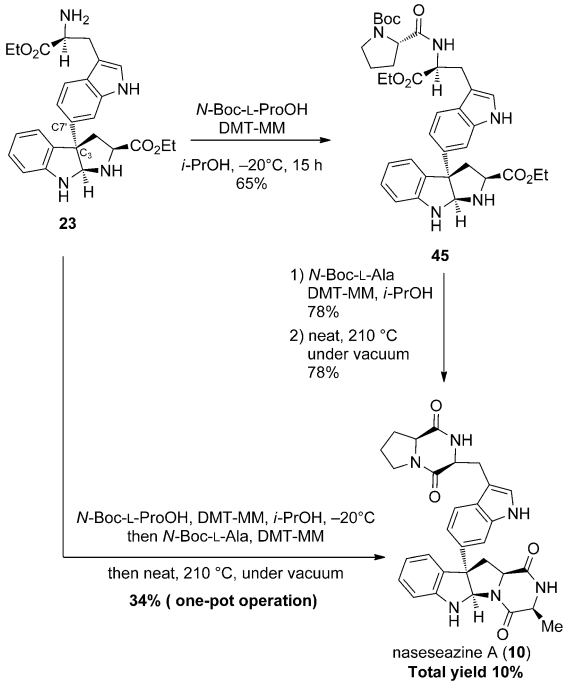


Scheme 10. Total synthesis of WIN 64745 (**7**), cristatumin C (**8**), and asperdimin (**9**).

dimin (**9**), *N*-Cbz-D-leucine and *N*-Cbz-D-valine were employed in the coupling reaction. Final diketopiperazine formation was carried out with 28% aqueous ammonia solution because diketopiperazine formation with D-amino acids is much easier than with L-amino acids. The chemical yield of asperdimin (**9**) was 71% in the one-pot procedure and the total yield was 20%. In addition, the yield was improved by using the one-pot protocol compared with the monoamidation reaction (Table 4, entry 4) because the purification with silica gel was avoided after monoamide formation. The obtained heterodimeric natural products through two one-pot procedures were identified by using the reported analytical data.

Synthesis of naseseazine A and naseseazine B

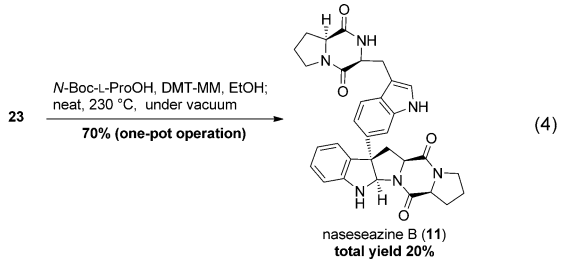
In 2009, naseseazine A (**10**) and naseseazine B (**11**) possessing a unique C3(sp^3)–C7(sp^2) bridge were isolated from Fijian actinomycete *Streptomyces sp*. The stereochemistry of these alkaloids was determined based on C_3 Marfey's analysis of its degradation products by Capon and co-workers.[11a] In 2011, total syntheses of naseseazine A (**10**) and naseseazine B (**11**) with a revision of their absolute stereochemistry were achieved by Kim and Movassaghi.[11b] The biosynthetic pathways of naseseazine A (**10**) and naseseazine B (**11**) were proposed as an oxidative cationic coupling sequence by Capon et al.,[11a] followed by a Friedel–Crafts-type coupling sequence through a single-electron oxidation by Movassaghi et al.[11b] Naseseazine A (**10**) contains an L-alanine unit on the lower indoline part and an L-proline unit on the upper indole part of the heterodimeric compound **23**. Therefore, the synthesis of compound **10** started from a selective condensation reaction of the primary amine on the upper indole part with *N*-Boc-L-proline (Scheme 11). Thus, compound **23** was treated with one equiva-



Scheme 11. Total synthesis of naseseazine A.

lent of *N*-Boc-L-proline in the presence of 1.1 equivalents of DMT-MM (*i*PrOH, −20 °C, 15 h) to provide the monoamide product **45** on the primary amine in good yield (65%), together with the undesired bisamide compound (13%). The second amidation and diketopiperazine formation of compound **45** were accomplished by treatment of one equivalent of *N*-Boc-L-Ala with DMT-MM (1.2 equiv, *i*PrOH, 0 °C, 9 h, 78%) followed by simple heating without solvent (neat, 210 °C, 0.1 mbar, 1 h, 78%) to provide naseseazine A (**10**). After establishing the separate synthetic protocols, we moved to a one-pot conversion from compound **23** to compound **10**. After initial amidation with *N*-Boc-L-proline (1.0 equiv) in the presence of DMT-MM (1.1 equiv), *N*-Boc-L-alanine (1.1 equiv) and additional DMT-MM (1.1 equiv) for the condensation to the secondary amine on the pyrrolidinoindoline core were added to the reaction mixture. When the second amidation was completed, the solvent was removed under reduced pressure followed by direct heating at 210 °C while maintaining the vacuum condition (0.1 mbar, 1 h) to provide naseseazine A (**10**) in moderate yield (34%). To summarize the synthesis of compound **10**, it was accomplished in a two-pot operation from compound **20** in a total yield of 10% by using $Mn(OAc)_3$ or VOF_3 oxidation in aqueous CH_3SO_3H solution (Table 2, entries 3 and 9).

The synthesis of naseseazine B (**11**) was accomplished by a one-pot procedure with *N*-Boc-L-proline (2.2 equiv) in the presence of DMT-MM [Eq. (4)]. Thus, the dehydration condensation reaction with **23** (1 equiv) and *N*-Boc-L-proline (2 equiv) in the presence of DMT-MM (2 equiv, EtOH, 0 °C, 4 h) was followed by diketopiperazine formation (neat, 230 °C, 15 min, 0.1 mbar) to provide **11** in superb yield (70%). Total yield of **11** was 20% via two one-pot operations.



(4)

Biological study

All synthetic alkaloids were examined for biological studies, including ubiquitin-specific protease 7 (USP7) inhibition, inhibition of foam cell formation in macrophages, and cytotoxicity (Table 5). The ubiquitin-proteasome system is an important pathway that regulates various cellular events through selective protein degradation,[27] and has emerged as a potential target for therapeutic intervention for cancer, inflammatory disorders, and neurodegenerative diseases. USP7 is one of the deubiquitinating enzymes and has emerged as an oncology target. USP7 stabilizes Mdm2, a major E3 for the tumor suppressor protein p53, through deubiquitinating auto-ubiquitinated Mdm2. Therefore, USP7 inhibitors are capable of stabilizing p53 in cells, which leads to the suppression of cancer.[28] Thus, selective inhibitors of USP7 are attractive drug candidates for cancer therapy.[29] The formation of foam cells in macrophages plays an essential role in the progression of early atherosclerotic lesions.[30] Foam cell formation is induced by acetylated low-density lipoproteins in human monocyte-derived macrophages and is characterized by the intracellular accumulation of cholesterol ester (CE).[31] Therefore, the inhibitors of CE accumulation become promising drug candidates for the prevention and treatment of atherosclerosis.[32] The evaluation of biological assays was carried out by using the compounds synthesized in this study, that is, the synthetic alkaloids (compounds **1**–**11**, Figure 1), the key tryptophan dimers (compounds **21**–**24**, Table 2), and the ditryptoleucine analogues (compounds **36**–**38**, Figure 3) at concentrations of 10 μM (Table 5). The USP7 inhibitory activities of 1′-(2-phenylethylene)-ditryptophenaline (**5**), WIN 64745 (**7**), and 15,15′-bis-*epi*-eurocristatine (**3**) (90, 77, and 54% inhibition at 10 μM, respectively, Table 5) were more potent than those of the other compounds. The presence of a phenylethylene group at the C-1′-position in the most potent inhibitor **5** may be important for USP7 inhibition, because ditryptophenaline (**4**), which lacks the functional group, showed no inhibition. Although compound **7**, WIN 64821 (**1**), and the ditryptoleucine analogue **36** (77, 15, and 1%, respectively, Table 5) contain the same skeleton, only compound **7** inhibited significantly. This result clearly indicated that the presence of the L-phenylalanine and L-leucine units at the C-15- and C-15′-positions was essential for USP7 inhibition in this skeleton. Among the compounds tested, only WIN 64745 (**7**) inhibited foam cell formation in macrophages (70% inhibition, Table 5). It is noteworthy that none of the compounds tested in this study showed cytotoxicity against HeLa cells at a concentration of 10 μM. Therefore, compounds **3**, **5**, and **7** are definitely attractive drug candidates for cancer

Table 5. Biological activities of the synthetic tryptophan-based diketopiperazine alkaloids.

Compound	USP7 inhibition [%][a]	Inhibition of foam cell formation in macrophages [%][a]
WIN 64821 (**1**)	15	0
eurocristatine (**2**)	7	0
15,15′-bis-*epi*-eurocristatine (**3**)	54	0
ditryptophenaline (**4**)	5	0
1′-(2-phenylethylene)-ditryptophenaline (**5**)	90	0
ditryptoleucin A (**6**)	2	0
WIN 64745 (**7**)	77	70
cristatumin C (**8**)	4	0
asperdimin (**9**)	6	0
naseseazine A (**10**)	2	0
naseseazine B (**11**)	8	0
dimeric compound **21**	0	0
dimeric compound **22**	0	0
dimeric compound **23**	8	0
dimeric compound **24**	6	0
dimeric compound **36**	1	0
dimeric compound **37**	0	0
dimeric compound **38**	0	0

[a] Tested at a concentration of 10 μM.

or atherosclerosis therapy. Studies of structure–activity relationships with the tryptophan-based diketopiperazine alkaloid library prepared by our two one-pot strategies are currently under way.

Conclusion

In conclusion, we have developed direct bioinspired dimerization reactions along with our proposed biosynthetic pathway from commercially available amine-free tryptophan derivatives in aqueous acidic media to provide C_2-symmetric and non-symmetric dimeric compounds. Then, concise two one-pot syntheses of the dimeric diketopiperazine alkaloids WIN 64821 (**1**), eurocristatine (**2**), 15,15′-bis-*epi*-eurocristatine (**3**), ditryptophenaline (**4**), ditryptoleucine A (**6**), WIN 64745 (**7**), cristatumin C (**8**), asperdimin (**9**), naseseazine A (**10**), and naseseazine B (**11**) were accomplished. In addition, 1′-(2-phenylethylene)-ditryptophenaline (**5**) was synthesized by three one-pot operations. The present synthesis has several noteworthy features. 1) Tryptophan-based C_2-symmetric and non-symmetric dimeric key intermediates can be prepared on a multigram scale in one step. 2) The developed oxidation reaction was carried out in acidic aqueous solution without deactivation or activation of the metal oxidants (i.e., $Mn(OAc)_3$, $CeSO_4$, VOF_3, and V_2O_5). 3) Protection of the primary amine can be avoided by salt formation in acidic water in the synthesis scheme. 4) Satisfactory total yields (10–27%) were obtained compared with previous reported syntheses. 5) Effective one-pot diketopiperazine formation was discovered by using the Kunishima coupling protocol followed by Boc deprotection and intramolecular amide formation under vacuum or COMU coupling followed by hydrogenation and base treatment. In addition, CD spectra of all synthetic natural products and intermediates were obtained (for details see the Supporting Information). This chiral information will be very helpful for the structure elucidation of unknown natural products in future.

The obtained synthetic libraries of naturally occurring diketopiperazine alkaloids and related compounds were evaluated with biological assays, including an USP7 inhibitory assay, inhibition of macrophage formation, and cytotoxicity. Finally, 15,15′-bis-*epi*-eurocristatine (**3**), 1′-(2-phenylethylene)-ditryptophenaline (**5**), and WIN 64745 (**7**) were discovered as new drug candidates for cancer therapy through USP7 inhibition. In addition, WIN 64745 (**7**) was newly listed as a drug candidate for atherosclerosis therapy through inhibition of macrophage formation.

Further applications of the bioinspired dimerization reactions, the one-pot diketopiperazine formation, and analogue syntheses followed by evaluation of biological studies for structure–activity relationships are currently under way.

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Keywords: alkaloids · amino acids · bioinspired reaction · dimerization · one-pot reactions

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[22] The geometry optimizations of compound **20** was carried out by using the DFT method, where the B3PW91 functional was used for the exchange-correlation term. Analytical vibrational frequency computations at the optimized structure were then performed to confirm that the optimized structure was at an energy minimum. The 6-311 + G(d,p) basis sets were employed in these calculations. The calculations were performed by using the Gaussian 09 program. a) Gaussian 09, Revision C.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian Inc., Wallingford CT, **2009**; b) R. Krishnan, J. S. Binkley, R. A. Seeger, J. A. Pople, *J. Chem. Phys.* **1980**, *72*, 650 – 654; c) M. J. Frisch, J. A. Pople, J. S. Binkley, *J. Chem. Phys.* **1984**, *80*, 3265 – 3269; d) T. Clark, J. Chandrasekhar, G. W. Spitznagel, P. v. R. Schleyer, *J. Coput. Chem.* **1983**, *4*, 294 – 301.

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